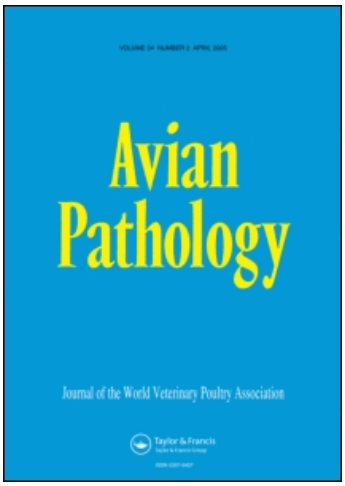

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Heat inactivation of avian influenza and Newcastle disease viruses in egg products

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Avian influenza (AI) and Newcastle disease (ND) viruses are heat labile viruses, but exact parameters for heat inactivation at egg pasteurization temperatures have not been established. In this study we artificially infected four egg products with two AI (one low [LP] and one high pathogenicity [HP]) and three ND (two low and one highly virulent) viruses, and determined inactivation curves at 55, 57, 59, 61 and 63°C. Based on D_t values, the time to inactivation of the viruses was dependent on virus strain and egg product, and was directly related to virus titre, but inversely related to temperature. For all temperatures, the five viruses had the most rapid and complete inactivation in 10% salt yolk, while the most resistant to inactivation was HPAI virus in dried egg white. This study demonstrated that the LPAI and all ND viruses were inactivated in all egg products when treated using industry standard pasteurization protocols. By contrast, the HPAI virus was inactivated in liquid egg products but not in dried egg whites when using the low-temperature industry pasteurization protocol.

Introduction

For 2002, the forecast world production of eggs for hatching or food consumption was 819 billion eggs (US Poultry and Egg Export Council, J. Geck, personal communication). From this production, an excess of $322 million in value of processed egg products were traded internationally. Each importing country requires assurances that such products are free from specific infectious agents, which could severely affect their nation's poultry health should they be introduced into the commercial production systems (Office International des Epizooties [OIE], 2003b). The World Organization for Animal Health (OIE) establishes the health standards for international trade in animals and animal products under the auspices of the World Trade Organization. The OIE considers avian influenza (AI) viruses of high pathogenicity (HP), and Newcastle disease (ND) viruses of the mesogenic and velogenic pathotypes as diseases of great significance that affect poultry (OIE, 2003a).

Provisions for importation of poultry products from AI-infected or ND-infected countries or infected compartments in countries can be made if they are processed in such a way as to inactivate the pathogens (OIE, 2003b). Heat application is the most common procedure used to inactivate infectious agents in meat and egg products through cooking and pasteurization, respectively. For poultry pathogens, previous studies have established heat inactivation curves for infectious bursal disease virus in cloacal bursal homogenate and velogenic ND virus in meat products (Alexander & Chettle, 1998; Alexander & Manvell, 2004).

AI virus is a heat-labile single-stranded RNA virus of the family Orthomyxoviridae, genus *Influenzavirus A*, while ND virus is a single-stranded RNA virus of the family Paramyxoviridae, genus *Avulavirus*, serogroup avian paramyxovirus 1 (APMV-1) (International Committee on Taxonomy of Viruses, 2002). Both AI and ND viruses can cause mild to severe disease in commercial poultry, including egg laying chickens, with virus being shed from the respiratory tracts and sometimes faeces (Alexander, 2003; Swayne & Halvorson, 2003). Lesions have been reported in the ovaries and

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oviducts of infected egg-laying chickens. Therefore, AI and ND viruses could potentially be transmitted via the egg either through virus in the internal egg contents or on the surface from virus-infected faeces. Both viruses can be partially protected from heat inactivation by the presence of organic material (Alexander, 2003; Swayne & Halvorson, 2003). In addition, some strains of ND virus have greater thermostability for haemagglutinin activity than AI viruses and the lentogenic B1, LaSota and derivative ND vaccine strains (King, 2001).

The present study was undertaken to determine the thermal inactivation curves of low pathogenicity (LP) and HPAI, and ND viruses in four artificially infected egg products. These four egg products were selected because they were used commercially and traded internationally.

Materials and Methods

Viruses. Two North American AI viruses were used: LP A/chicken/New York/13142-5/94 (H7N2) (LPAI/NY/94) and HP A/chicken/Pennsylvania/1370/83 (H5N2) (HPAI/PA/83) (Suarez *et al*., 1999; Davison *et al*., 2003). Three ND virus strains were used: lentogenic Ulster (NDV/Ulster), lentogenic B1 vaccine (NDV/B1), and velogenic California/02 (NDV/CA02) ND viral strains. Previous studies had demonstrated NDV/Ulster strain had greater thermostability than NDV/B1 (King, 1991). All the viruses were propagated by allantoic sac inoculation of 9-day-old embryonating chicken eggs by standard methods (Swayne *et al*., 1998; Alexander, 1998).

Testing method development. Samples of homogenized whole egg (0.4 ml) artificially infected with LPAI/NY/94 ($10^{5.7}$ median embryo infectious doses [EID_{50}]/ml) were placed in 12 ml screw-cap, flat-bottom glass vials (Glass Vial #225145; Wheaton, Millville, NJ, USA) and exposed to 55, 57, 59, 61 or 63°C in a precision waterbath (Versa-Bath, # 15-458-106; Fisher Scientific, Pittsburg, PA, USA) for 1, 2, 3, 4, 6, 8 and 12 min. Similarly, 80 µl homogenized whole egg artificially infected with LPAI/NY/94 ($10^{5.3}$ EID_{50}/ml) was placed in 200 µl sealable snap-top, thin walled thermocycler tubes (Thermowell Tubes® # 6531; Corning Inc., Corning, NY, USA) and exposed to 55, 57, 59, 61 or 63°C in a precision thermocycler (GeneAmp PCR System 2400; Perkin-Elmer, Boston, MA, USA). The samples were then diluted 1:10 in phosphate-buffered saline and inoculation into 9-day-old embryonating chicken eggs by standard methods to identify infectious virus (Swayne *et al*., 1998).

Experimental design—final testing method. Each virus was added to four different egg products (homogenized whole egg, liquid egg white, 10% salted egg yolk [Michael Foods, Lenox, IA, USA] and dried egg white [Michael Foods, Wakefield, NA, USA]) to achieve a final concentration of approximately $10^{5.5}$ EID_{50}/ml product (actual back titres ranged from $10^{4.5}$ to $10^{6.4}$ EID_{50}/ml at the zero time point; unpublished data). For the first three products, infective allantoic fluid was added to each product and gently vortexed until mixed. Then 80 µl was placed in 200 µl thin-walled thermocycler tubes and held at 4°C until tested in precision thermocyclers (PTC-200 DNA Engine; MJ Research, Waltham, MA, USA). For the dried egg whites, the product was rehydrated in sterile water to achieve 82% protein concentration and virus was added. Then 80 µl was dispensed into the thermocycler tubes and freeze-dried prior to heat treatment in the thermocyclers (PTC-200 DNA Engine and PTC 150 MiniCycler; MJ Research; and iCycler; BioRad, Hercules, CA, USA).

For determining thermal inactivation, the thermocycler was programmed to begin timing after the specified volume of sample reached the desired temperature. Once placed in the thermocycler, homogenized whole egg, liquid egg white and 10% salted egg yolk samples were removed at 1, 2, 3, 4, 6, 8 and 12 min, and dried egg white samples were removed at 1, 2, 3, 5, 7 and 10 days, and immediately chilled in an ice bath. Each sampling time was performed in triplicate.

Homogenized whole egg, liquid egg white and salted yolk samples were processed immediately for virus isolation and titration in embryonating chicken eggs (Alexander, 1998; Swayne *et al*., 1998). Dried egg white samples were stored at −70°C until assayed. The Reed and Muench method was used to calculate the end points and results were recorded as EID_{50} per millilitre (Reed & Muench, 1938).

Statistics and graphics. To evaluate the effect of pasteurization on inactivation of viruses in egg products, three values were calculated: (1) D_t, the time to reduce the infectious titre by 10^1 EID_{50} at a specified temperature; (2) Max D_t, the maximum D_t value for samples with lack of infectious virus detection at the first sampling time; and (3) Z, temperature (°C) increase necessary to reduce the D_t value a log cycle.

To calculate D_t for each egg product at each temperature, the $\log_{10}$ average infectious titre of the three replicates with standard deviation was plotted against time as a line graph, and a linear trendline line (least-squares fit), line equation and R^2 value were calculated (Excel®; Microsoft, Seattle, WA, USA). Triplicate data sets with only one sample point were discarded while data sets with two sample points were used, but a third data point, the next lower detection limit with addition of another egg to the virus isolation assay ($10^{1.9}$ EID_{50}), was added to obtain the average infectious titre. To calculate Z values, the D_t values ($\log_{10}$) were plotted against the temperatures (°C) as a line graph, and a linear trendline line (least-squares fit), line equation and R^2 value were calculated (Excel®).

Formulas used were:

$$D_t = \left[\left(\frac{1 - L_t}{S_t}\right) - \left(\frac{2 - L_t}{S_t}\right)\right] \qquad \text{Max } D_t = \frac{T}{V_1 - 1.97}$$

$$Z = \left[\left(\frac{1 - I_d}{S_d}\right) - \left(\frac{2 - I_d}{S_d}\right)\right]$$

where t is temperature, I_t is y-axis intercept and S_t is slope from the linear trendline equation (infectious titre [$\log_{10}$] verses time), T is time point of first sample, V_1 is mean titre ($\log_{10}$ EID_{50}/ml) of the virus at time zero, and 1.97 is lowest detection limit ($\log_{10}$) of the virus isolation test ($\log_{10}$ EID_{50}/ml), and I_d is y-axis intercept and S_d is slope from the linear trendline equation (D_t verses temperature).

Results

Testing method development. Use of flat-bottom glass vials in the precision water bath method resulted in inactivation of LPAI/NY/94 in homogenized whole egg at 57, 59, 61 and 63°C, but this inactivation was not consistent with the length of time for the heat treatment (Table 1). By contrast, use of 200 µl plastic vials and thermocycler heat blocks resulted in predictable inactivation of the LPAI virus at 57, 59, 61 and 63°C, which was directly and consistently dependent upon the length of time of the heat treatment (Table 1). Neither method demonstrated virus inactivation at 55°C. For the final inactivation study, only the thermocycler method was used.

Inactivation curves and D_t values. Plotting the $\log_{10}$ titre verses time generated three different types of inactivation curves: (1) Biphasic A, (2) Biphasic B, and (3) Monophasic (Figure 1). Biphasic A began with an immediate decline in titre from the zero time point with two inactivation curves: the first curve having a steeper slope than the second curve (Figure 1). For determination of the D_t values, only

Table 1. *Comparison of inactivation of LPAI/NY/94 in artificially infected homogenized whole egg by water bath versus thermocycler methods*

		Time (min)							
Method	Temperature (°C)	0	1	2	3	4	6	8	12
Water bath	55	+	+	+	+	+	+	+	+
	57	+	+	+	−	−	+	+	−
	59	+	+	+	−	−	−	−	+
	61	+	+	−	+	−	−	−	+
	63	+	−	−	−	+	+	+	−
Thermocycler	55	+	+	+	+	+	+	+	+
	57	+	+	+	+	−	−	−	−
	59	+	+	−	−	−	−	−	−
	61	+	+	−	−	−	−	−	−
	63	+	−	−	−	−	−	−	−

+, positive for avian influenza virus; −, negative for influenza virus.

the second curve was used. For the Biphasic B, the 0 min, 1 min and, sometimes, 2 min time points were a plateau that was followed by a linear decline in titre (Figure 1). The Monophasic had a single linear curve beginning with the zero time point.

The time for inactivation of the viruses, based on D_t values, was directly related to virus titre and inversely related to temperature (Table 2). In addition, inactivation for each virus varied depending upon the egg medium in which it was contained. Viruses in the 10% salt yolk had the most rapid and complete inactivation for both AI and ND viruses. By contrast, the most resistance to inactivation was HPAI virus in dried egg whites.

Fifty-eight of the 100 sample points, representing various combinations of the five viruses, five temperatures and four egg products, lacked detection of any virus at the first sampling time point; that is, either 1 min (other three egg products) or 1 day (dried egg white) (Table 2). For these 58 sample points, calculation of D_t values was not possible and thus Max D_t values were reported (Table 2). Max D_t values were used for 21 of 25 10% salted egg yolk, 15 of 25 dried egg white, 12 of 25 egg white, and 10 of 25 homogenized whole egg sample points.

***Z* values**. When examining each individual egg product, the ND viruses were more resistant to heat inactivation as evidenced by greater Z values than were the AI viruses (Table 3). When comparing egg products, the greatest resistance to heat inactivation (higher Z values) was seen in dried egg whites, followed by homogenized whole egg. Liquid egg white had the lowest Z values while 10% salted yolk had insufficient data to draw conclusions.

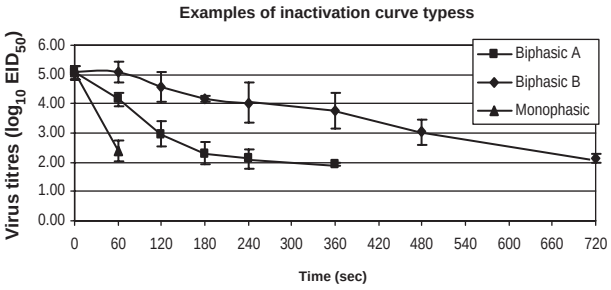

Figure 1. *Example of the three types of inactivation curves (55 to 63°C) seen in this study. Example was for NDV/B1 in homogenized whole egg.*

Pasteurization to inactivate virus at levels reported in eggs. The literature does not contain data on levels of ND and LPAI viruses within eggs laid by infected hens, thus no additional calculations were carried out. However, for HPAI/PA/83 virus, the D_t values ($\log_{10}$) in Table 2 were plotted against temperature for each egg product (Figure 2), and, using the resulting line equations, specific D_t values were calculated for each egg product at industry standard pasteurization temperatures (Table 4). In homogenized whole egg, liquid egg white and 10% salted egg yolk, the time periods to inactivate HPAI/PA/83 from $10^{4.9}$ EID_{50} HPAI virus/ml (the maximum titre in eggs laid by HPAI/PA/83 infected hens; M. Brugh, unpublished data) to 10^0 EID_{50} HPAI virus/ml (titre insufficient to infect chickens with a similar H5N2 HPAI virus, A/chicken/Queretero/14588/95; Swayne, 2003) required less time than is used in the industry standard pasteurization protocols (Table 4). In addition, an extra margin of safety was added in Table 4 by calculations for the reduction of final virus concentrations to 10^{-1} and 10^{-2} EID_{50}/ml (i.e. 1:10 and 1:100 chance of 10^0 EID_{50}, respectively). One industry pasteurization protocol (54.4°C for 7 to 10 days) was not effective for inactivation of HPAI virus in dried egg white (Table 4).

Discussion

Pasteurization of egg products has been practiced since the 1930s primarily as a food safety measure to inactivate contaminating *Salmonella* obtained from the faeces on the eggshell surface or from the

Table 2. D_t *values (time to reduce virus titre by 90% or* 10^1 EID_{50}*), and curve type for four egg products artificially infected with AI and ND viruses*

Virus	Temperature (°C)	Egg products			
		Homogenized whole egg (sec)	Liquid egg white (sec)	10% salted yolk (sec)	Dried egg white (days)
LPAI/NY/H7N2	55	401.1 (BiA)	396 (BiA)	40.8 (M)	0.5 (M)
	57	134.9 (BiA)	21.3 (M)	22 (M)	<0.4
	59	21.8 (M)	<19	<20	<0.4
	61	<20	<19	<20	<0.4
	63	<20	<19	<20	<0.4
HPAI/PA/H5N2	55	643.8 (BiB)	256.7 (BiA)	20.3 (M)	2.2 (BiA)
	57	268.5 (BiA)	22.9 (M)	<20	1.4 (BiA)
	59	22.3 (M)	<19	<20	1.3 (BiA)
	61	<19	<19	<20	1.0 (BiA)
	63	<19	<19	<20	0.2 (M)
NDV/Ulster	55	320.7 (BiB)	282.8 (BiA)	<19	<0.25
	57	228 (BiA)	89.7 (BiA)	<19	<0.25
	59	96.3 (M)	43 (M)	<19	<0.25
	61	<18	<20	<19	<0.25
	63	<18	<20	<19	<0.25
NDV/B1	55	237.7 (BiB)	260.9 (BiA)	25.2 (M)	0.3 (M)
	57	88.3 (BiA)	105.4 (BiA)	<24	0.3 (M)
	59	22.4 (M)	21.7 (M)	<24	<0.27
	61	<18	<19	<24	<0.27
	63	<18	<19	<24	<0.27
NDV/CA/02	55	360.1 (BiB)	325.4 (BiB)	<18	0.3 (M)
	57	160.7 (M)	140.8 (BiA)	<18	0.2 (M)
	59	57.6 (M)	40.3 (M)	<18	<0.23
	61	<20	<19	<18	<0.23
	63	<20	<19	<18	<0.23

BiA, Biphasic A; BiB, Biphasic B; M, Monophasic (see Figure 1).

internal contents of the egg (Froning *et al*., 2002). Various minimum standards have been published for pasteurization of egg products including liquid albumen (56.7°C, 3.5 min), whole egg (60°C, 3.5 min), 10% salted egg yolk (63.3°C, 3.5 min) and dried egg white (54.4°C, 7 to 10 days). Recently, new standards have been proposed for the inactivation of *Salmonella* in 10% salted yolk (63.3°C, 4.5 min) and liquid egg whites (57.7°C, 6.3 min) (Froning *et al*., 2002). A side benefit of pasteurization could be inactivation of additional bacterial species as well as various viruses or fungi that could affect human or possibly animal health should egg products be included as a nutrient source in uncooked foods or feeds. From the current studies, we have demonstrated that heat application to egg products artificially infected with low or high virulent AI or ND viruses can result in virus inactivation when using temperatures and times of heat application similar to those used in commercial pasteurization of liquid egg products.

Previous limited studies have demonstrated the concept of heat to inactivate AI and ND viruses in artificially infected egg contents. In one study, NDV/Ulster, NDV/California/1083/72 and HPAI/PA/83 were tested for 5, 10, 15, 30, 40, 50 and 60 min at 57°C in artificially infected yolk and liquid albumen samples using screw-cap glass vials and a water bath (King, 1991). In albumen samples, $10^{5.2}$ EID_{50}/ml AI virus was inactivated between 5 and 10 min, but the NDV/Ulster ($10^{7.1}$ EID_{50}/ml) and NDV/California/1083/72 ($10^{7.9}$ EID_{50}/ml) ND

Table 3. *Z values (temperature [°C] increase necessary to reduce* D_t *value a log cycle) for four egg products artificially infected with five different AI and ND viruses*

Virus	Egg products			
	Homogenized whole egg	Liquid egg white	10% salted yolk	Dried egg white
LPAI/NY/94	3.2	1.6	7.5	–
HPAI/PA/03	3.6	1.9	–[a]	9
NDV/Ulster	7.7	4.9	–	–
NDV/B1	3.9	3.7	–	–
NDV/CA/02	5.0	4.4	–	11.4

[a] –, inadequate data to calculate value.

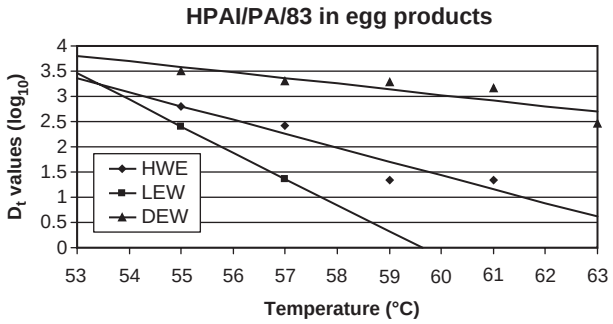

Figure 2. *The plot of* D_t *values* (log_{10}) *against temperature* (°C) *for HPAI/PA/83 virus in homogenized whole egg (HWE), liquid egg white (LEW) and dried egg white (DEW) from Table 2. Values for 10% salted yolk were Max* D_t *value at 57°C and above. Trendline equations were used with* x = *temperature* (°C) *and resulting* y = *time (log10). HWE:* $y = (-0.274[x - 52°C]) + 3.626$. *LEW:* $y = (-0.5248[x - 52°C]) + 3.9838$. *DEW:* $y = (-0.1114[x - 52°C]) + 0.7608$.

viruses required greater than 40 and 50 min treatment for inactivation, respectively. For yolk, the two ND viruses were not inactivated during the 1 h treatment period. In another study, heat inactivation was demonstrated for two velogenic ND viruses, Herts/33 and Beaudette C, artificially added to liquid whole egg and exposed to 64.4°C, which followed a bi-kinetic curve (Gough, 1973). Following re-examination of the original data, the D_t value for Beaudette C was extrapolated to be 38 sec (Alexander & Chettle, 1998). By comparison, the analogous portion of the current study (artificially infected homogenized whole egg, heat treated at 63°C), LPAI/NY/94, HPAI/PA/83, NDV/Ulster, NDV/B1 and NDV/CA/02 had D_t values consisting of Max D_t values <20, <19, <18 sec, <18 and <20 sec, respectively. Our inactivation studies may have been more precise than previous studies because of our use of thin-walled, small volume plastic tubes and precision thermocycler plates versus less consistent results from preliminary studies that used a traditional thick-walled large volume glass vial/water bath method. Previous studies with Salmonella inactivation have shown small glass capillary tubes to give more accurate results than large glass tubes because the smaller tubes have instant come-up and cool-down times (Schuman *et al*., 1997).

From the current study, inactivation of AI and ND viruses in the egg products was dependent on type of egg product, virus strain, temperature of process, length of treatment and concentration of virus. After deriving the D_t values, determination of the virus concentration in eggs laid by infected chickens is the final step in assessing the effectiveness of pasteurization for inactivation of AI and ND viruses in egg products. During the 1983/84 HPAI outbreak in the northeastern United States, AI virus was isolated from albumen and yolk samples and the shell surface of eggs obtained from broiler breeder and layer flocks in Pennsylvania with infection rates ranging from 7% to 57% of the eggs laid between 1 and 18 days after the appearance of clinical signs (Cappucci, Jr., *et al*., 1985). In addition, HPAI virus has been isolated from internal contents of eggs laid by experimentally infected chickens and turkeys (Moses *et al*., 1948; Narayan *et al*., 1969; Beard *et al*., 1984). In one experimental study with HPAI/PA/83 in hens, AI virus was present in 85 to 100% of eggs laid on days 3 and 4 post inoculation (p.i.), with concentration as high as $10^{4.9}$ EID_{50} HPAI virus/ml egg product (M. Brugh, unpublished data), while AI virus was not present in eggs laid on days 1 and 2 p.i. (Beard *et al*., 1984). Although the $10^{4.9}$ EID_{50} HPAI virus/ml level was based on a small number of eggs, standard industry

Table 4. *Estimation of pasteurization times for egg products obtained from eggs laid by HPAI/PA/83 infected hens and based on* D_t *values of current study*

	Industry pasteurization standards[a]				Time to inactivate HPAI in eggs		
Egg product	Temperature (°C)	Time	D_t values calculated from Figure 2	Maximum titre of HPAI virus in egg (log_{10} EID_{50}/ml)	0 log_{10}[b] EID_{50}/ml	−1 log_{10}[c] EID_{50}/ml	−2 log_{10}[d] EID_{50}/ml
Whole egg	60	210 sec	27.2 sec	4.9	133 sec	160 sec	188 sec
Whole egg blends	60	372 sec	27.2 sec	4.9	133 sec	160 sec	188 sec
Whole egg blends	61.1	210 sec	13.6 sec	4.9	67 sec	80 sec	94 sec
Liquid egg white	55.6	372 sec	37.1 sec	4.9	182 sec	219 sec	256 sec
Liquid egg white	56.7	210 sec	33.1 sec	4.9	162 sec	195 sec	228 sec
10% Salted yolk	62.2	372 sec	<20 sec	4.9	<98 sec	<118 sec	<138 sec
10% Salted yolk	63.3	210 sec	<20 sec	4.9	<98 sec	<118 sec	<138 sec
Dried egg white	54.4	7 to 10 days	3.1 days	4.9	15.2 days	18.3 days	21.38 days
Dried egg white	67	15 days	0.12 days	4.9	0.59 days	0.71 days	0.83 days

[a] Froning *et al*. (2002).
[b] Inactivation to level of 10^0 EID50/ml, which was below the dose that can infect chickens with A/chicken/Queretero/14588/95 (H5N2) HPAI virus (Swayne, 2003).
[c] Probability of 1 in 10 of having 1 EID_{50}/ml.
[d] Probability of 1 in 100 of having 1 EID_{50}/ml.

pasteurization protocols were effective for inactivation at such a level in homogenized whole egg, liquid egg white and 10% salted egg yolk (Table 4). However, for dried egg white, the inactivation time of 15.2 days at 54.4°C exceeded the industry standard of 7 days and would be inadequate for commercial application. Recently, a study demonstrated that treatment of dried egg white products at 67°C for 15 days resulted in improved egg functional properties, complete inactivation of high levels of *Salmonella* and preservation of bacteriostatic properties (Baron *et al*., 2003). Treatment of dried egg whites at 67°C for 15 days would be effective for inactivating HPAI virus, but still maintain quality properties of egg white. Alternatively, pasteurization of liquid egg white before use in the production of the dried egg white would be an effective HPAI virus inactivation strategy. Additional experimental studies are needed to verify the quantity of HPAI virus in eggs laid by infected hens.

In contrast to HPAI viruses, no LPAI virus-positive albumen samples were identified in the 9930 eggs tested from three layer flocks infected with H7N2 LPAI virus during 1996 to 1998 in Pennsylvania (P. Dunn, personal communication). Likewise, no virus was isolated from shell swabs, albumen or yolk samples from 60 eggs taken from each of two broiler breeder farms 7 days after onset of clinical respiratory disease and drops in egg production in the 2001/02 outbreak of H7N2 LPAI virus (Lu *et al*., 2004). Virus was isolated from most pools of tracheal and cloacal swabs samples. In another study of the 1996 to 1998 H7N2 LPAI outbreak in Pennsylvania chickens, acute salpingitis with necrosis of epithelium in the albumen-secreting gland and isolation of virus from oviduct pools was commonly reported (Ziegler *et al*., 1999). These findings suggest LPAI virus has the potential to be deposited in eggs laid by acutely infected hens, but to date LPAI virus infected eggs have not been identified, which suggests the frequency of virus infection and the potential levels of virus in the such eggs may be low.

Little is know about deposition of ND virus in eggs. Several pre-1960 publications reported isolation of ND field and vaccinal viruses from dried eggs or content of eggs, but details are not available and in none of the studies was the amount of ND virus quantified (Gough, 1973). Isolation of vaccinal virus (lentogenic strains) from egg contents has not been reported since the 1960s. In recent years, velogenic ND virus has been sporadically identified in eggs of infected hens based on recovery of virus from hatched chicks or from cell cultures prepared from embryos of infected hens (Capua *et al*., 1993; Chen & Wang, 2002). Since velogenic ND virus can be heat inactivated at pasteurization temperatures and has been infrequently identified in eggs, this suggests that the pasteurization process could be effectively used to inactivate any ND virus present. Additional experimental studies are needed to assess the levels of ND virus in eggs. Also, the role that vaccination may play in preventing virus deposition in eggs needs to be assessed for both AI and ND viruses.

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